serum level of the bone turnover marker osteocalcin was significantly ($P < 0.001$) elevated in OVX and PTH treated compared to sham and vehicle treated, while the collagen type II degradation decreased, as the level of serum cartilage degradation marker CTX-II was reduced by 30% ($P < 0.01$), compared to vehicle treated OVX animals.
Conclusions: Human OA chondrocytes express the PTH1R receptor and PTH seems to induce an anabolic response in articular cartilage. Moreover, PTH had an anti-catabolic effect in the preclinical model of accelerated cartilage loss. Current results strongly suggest that PTH has direct beneficial effects on human OA chondrocytes and cartilage. Further research is necessary to investigate the potential of PTH as a disease modifying OA drug (DMOAD).

256
EFFECT OF DIACEREIN/RHEIN ON THE WNT SYSTEM IN HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE
J. Martel-Pelletier, F. Mineau, J. Caron, J-P. Pelletier. *Univ. of Montreal Hosp. Res. Ctr. (CRCHUM), Montreal, QC, Canada*

Purpose: The alterations in subchondral bone are an essential component of the structural changes that occur in osteoarthritis (OA). These include the undermineralization of the OA subchondral bone matrix, which is related to a significant increase in collagen type I alpha 1 (COL1A1). Previous studies have demonstrated that diacerein, an antirheumatic drug from the anthraquinone chemical class, improves OA subchondral bone metabolism and has a potential role in promoting homeostasis of this tissue in OA patients. The aim of the present study was to further investigate whether the mode of action of diacerein on OA subchondral bone could be mediated through an action on the Wnt system, more specifically on the canonical pathway β-catenin, the Wnt-1-induced secreted protein 1 (WISP-1), and the Wnt antagonists dickkopf (DKK)-1 and DKK-2. Moreover, we also studied the effect of diacerein on the level of COL1A1 and COL1A2.
Methods: Normal and OA human subchondral bone osteoblast basal expression levels (real time PCR) of β-catenin, WISP-1, DKK-1 and DKK-2 were evaluated. OA osteoblasts were cultured in a medium containing 5, 10 or 20 μg/ml diacerein and rhein (the active metabolite of diacerein) for 18 h, and the expression levels of the above factors as well as COL1A1 and COL1A2 were evaluated.
Results: The expression levels of β-catenin and WISP-1 were similar in normal and OA subchondral bone osteoblasts, whereas DKK-1 and DKK-2 were significantly increased ($p \leq 0.05$) in OA cells. Treatment with diacerein/rhein significantly reduced the expression level of COL1A1 at concentrations of 10 and 20 μg/ml ($p \leq 0.004$), whereas the level of COL1A2 remained unchanged. Interestingly, the ratio of COL1A1-to-COL1A2 decreased dose-dependently in diacerein treated cells. Moreover, the drugs significantly elevated the expression level of β-catenin ($p \leq 0.01$) and dose-dependently inhibited the Wnt antagonists DKK-1 and DKK-2 ($p \leq 0.009$). The WISP expression level was increased by diacerein/rhein at all concentrations tested.
Conclusion: These findings indicate that treatment with diacerein has a positive impact on OA subchondral bone osteoblasts by increasing the level of β-catenin, decreasing the levels of major inhibitors of the Wnt system, and curbing collagen type I formation, which, if translated in vivo, will have a potentially beneficial effect on mineralization of this OA tissue.
This study was funded in part by a grant from TRB Chemedica International S.A., Geneva, Switzerland.

257
ANALYSIS OF AUTOPHAGIC ACTIVITY IN HUMAN CARTILAGE AND CHONDROCYTES
H. Sasaki, T. Matsushita, K. Takayama, S. Kubo, T. Matsumoto, K. Ishida, S. Oka, M. Kurosaka, R. Kuroda. *Kobe Univ. Graduate Sch. of Med., Kobe, Japan*

Purpose: Autophagy, an evolutionarily conserved process for the bulk degradation of cytoplasmic components, has been reported to be a cell survival mechanism under starved conditions. The main functions of autophagy are housekeeping and quality control of proteins and organelles, therefore dysfunction of autophagy has been indicated as a cause of some degenerative diseases. However the role of autophagy in osteoarthritis (OA), the most common degenerative disease in human joints, has not yet been clear. The purpose of this study is to analyze autophagic activity in human chondrocytes and pathophysiology of OA.
Materials and Methods: OA articular cartilage was obtained from femoral condyles of patients with medial type OA during total knee arthroplasty. Lateral condyles and medial condyles were used as mild OA cartilage and severe OA cartilage respectively. Human non-OA cartilage was obtained from femoral heads of patients with femoral neck fracture and used as non-OA cartilage. Primary chondrocytes were also isolated from the articular cartilage samples of patients with OA and used as OA chondrocytes. Normal Human Articular Chondrocytes-knee (NHAC-kn) cells were purchased and used as normal human chondrocytes. The state of autophagy in the articular cartilage samples and the chondrocytes were assessed by immunohistochemistry and immunoblotting using antibodies for autophagy markers, LC3II and beclin1. In addition, we stimulated NHAC-kn cells with various stresses (IL-1β, NO, serum starvation) and examined the effects of the stresses on the autophagic activity by real-time PCR and immunoblotting.
Results: Immunohistochemical analysis showed that the expression of LC3 and beclin-1 were increased in the mild OA cartilage compared with the non-OA cartilage. On the other hand, the expression of LC3 and beclin-1 were decreased in the severe cartilage. LC3 and beclin1 were more strongly expressed in the superficial zone of the mild OA cartilage than in the deep zone and the middle zone. In addition, the primary OA chondrocytes strongly expressed LC3II and beclin-1 compared with NHAC-kn. Furthermore the expression of LC3 and beclin-1 in NHAC-kn were increased by the stresses.
Conclusions: In this study, we found that autophagy was increased in the mild OA cartilage. Autophagy was especially increased in the superficial zone presumably where chondrocytes were under more stresses compared with other zones. In addition, autophagy was increased in normal chondrocytes by the stresses *in vitro*. These observations suggested autophagic activity increases during early stage of OA and the increased autophagy was an adaptive response to protect cells from stresses. On the other hand, autophagy was decreased in the severe OA cartilage, suggesting that dysfunction of autophagy might be a cause of the progression of OA. Further studies about autophagy in chondrocytes will provide novel insights into the pathophysiology of OA.

258
EFFECT OF MECHANICAL STRESS OR ITS COMBINATION WITH MAPK INHIBITOR ON THE CHONDROCYTIC PHENOTYPE OF 3-DIMENSIONAL SCAFFOLD-EMBEDDED RAT CHONDROCYTES
S. Shioji. *Shiga Univ. of Med. Sci., Otsu, Japan*

Purpose: Chondrocytes play the major role in the homeostasis of articular cartilage, and they alter the activity of matrix synthesis in response to mechanical stress (MS).
A variety of signaling pathways have been implicated in the signaling process following the mechanical activation of chondrocytes. The signaling pathways related to mechanotransduction have been demonstrated to use the mitogen activated protein kinases (MAPKs) pathway, in which extracellular-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 constitute the signaling kinase. The mechanotransduction roles of ERK, JNK, and p38 pathway have remained controversial.
Since chondrocytes alter their metabolic activities according to their extracellular environments, we studied signaling pathways also using 3-dimensional (3D)-embedded chondrocytes. The present study attempts to characterize their responses to mechanical stress in terms of the extracellular and intracellular pathways of mechanotransduction.
Methods: Chondrocytes were isolated from rat articular cartilage. On reaching confluence, the cells were 3D-embedded in type 1 collagen scaffold. The cell-seeded scaffold was cultured either under mechanical stress (MS group). To investigate the involvement of second messenger pathway(s) of ERK, JNK and p38, the 3D-embedded chondrocytes were incubated in the presence or absence of the ERK inhibitor UO126, the JNK inhibitor SP600125, or the p38 inhibitor SB203580 at different concentration prior to MS. The mechanical stress was a cyclic compression at 5% compression, 0.33 Hz for 1hours. The 3D-embedded chondrocytes with neither mechanical stress nor MAPK inhibitor served as non-stressed (NS) cells. Real-time PCR was performed for Aggrecan (AGC), type 2 collagen (Col.2), and GAPDH at 1hour after the application of the mechanical stress.
Results: Expression of AGC and Col.2 was significantly upregulated in MS group when compared with that of the NS group. We then found that the